NOVEL USES OF NON-PEPTIDE BOMBESIN RECEPTOR ANTAGONISTS

BACKGROUND OF THE INVENTION

Thirty to 50 % of American women complain of sexual dysfunction. Ageing, menopause, and decline in circulating oestrogen levels significantly increase the incidence of sexual complaints. Evaluation of physiologic components of the female sexual response has, in the past, been technically challenging and difficult to standardise. In a recent publication (Berman J.R. et al. Clinical evaluation of female sexual function: effects of age and oestrogen status on subjective and physiologic sexual responses. Int. J. Impot. Res., 1999, 11: S31-38), the authors describe methodology for evaluating physiologic and subjective components of the female sexual response in the clinical setting and determine the effects of age and oestrogen status on them.

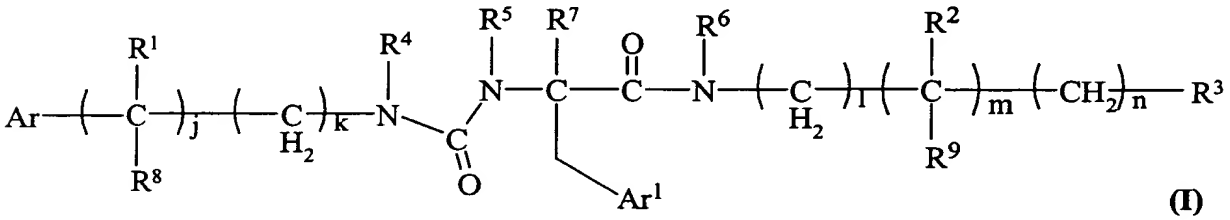
The amphibian tetradecapeptide bombesin (BB) belongs to a novel class of peptides that share structural homology within their C-terminal sequences (Dutta A.S. (1993) *in* Small Peptides: Chemistry, Biology, & Clinical Studies, Chapter 2, pp 66-82, Elsevier).

The decapeptides neuromedin B (NMB) and neuromedin C (NMC) and a 27 residue amino acid, gastrin-releasing peptide (GRP), are the three mammalian bombesin-like peptides to have thus far been identified (Battey J. *et al.* (1991), Trends Neurosci. **14**: 524). NMB and GRP are believed to mediate a variety of peripheral and centrally mediated biological actions by acting upon the corresponding NMB-preferring (BB_1) and GRP-preferring (BB_2) receptors (Lebacq-Verheyden A. *et al.* (1990) Handbook of Experimental Pharmacology **95** (Part II): 71). International patent application WO 98/07718 describes non-peptide bombesin receptor antagonists.

It has now been discovered that compounds described in WO 98/07718 and related compounds may be useful for the diagnosis, prevention, or treatment of sexual dysfunctions, anxiety and panic disorders, pulmonary hypertension, lung repair and lung development disorders, prostate cancer, pancreatic cancer, hepatic porphyria, visceral pain, gastrointestinal secretory disturbances, emesis or anorexia. The bombesin receptor antagonists utilised in the instant invention are described in the international patent application WO 98/07718 whose disclosure is incorporated herein by reference.

SUMMARY OF THE INVENTION

This invention therefore provides a method for preventing or treating various diseases amenable to therapy by a bombesin receptor antagonist, including male or female sexual dysfunctions, anxiety and panic disorders, social phobia, pulmonary hypertension, lung repair and lung development disorders, prostate cancer, pancreatic cancer, hepatic porphyria, visceral pain, gastrointestinal secretory disturbances, emesis or anorexia, inflammatory pain, neuropathic pain, cancer pain, postoperative pain, trigeminal neuralgia pain, acute herpetic and post herpetic pain, said method comprising administering to a patient in need of such treatment an effective amount of a bombesin receptor antagonist of Formula I



(I)

or a pharmaceutically acceptable salt thereof wherein

j is 0 or 1;

k is 0 or 1;

l is 0, 1, 2, or 3;

m is 0 or 1;

n is 0, 1 or 2;

Ar is phenyl, pyridyl or pyrimidyl, each unsubstituted or substituted by from 1 to 3 substituents selected from alkyl, halogen, alkoxy, acetyl, nitro, amino, $-CH_2NR^{10}R^{11}$, cyano, $-CF_3$, $-NHCONH_2$, and $-CO_2R^{12}$;

R^1 is hydrogen or straight, branched, or cyclic alkyl of from 1 to 7 carbon atoms;

R^8 is hydrogen or forms a ring with R^1 of from 3 to 7 carbon atoms;

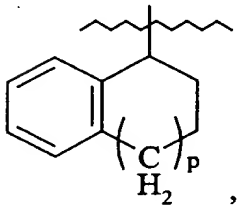
R^2 is hydrogen or straight, branched, or cyclic alkyl of from 1 to 8 carbon atoms which can also contain 1 to 2 oxygen or nitrogen atoms;

R^9 is hydrogen or forms a ring of from 3 to 7 carbon atoms with R^2 which can contain an oxygen or nitrogen atom; or R^2 and R^9 can together be a carbonyl;

Ar^1 can be independently selected from Ar and can also include pyridyl-N-oxide, indolyl, imidazolyl, and pyridyl;

R^4, R^5, R^6, and R^7 are each independently selected from hydrogen and lower alkyl; R^4 can also form with R^5 a covalent link of 2 to 3 atoms which may include an oxygen or a nitrogen atom;

R^3 can be independently selected from Ar or is hydrogen, hydroxy, $-NMe_2$, N-methyl-pyrrolyl, imidazolyl, N-methyl-imidazolyl, tetrazolyl, N-methyl-tetrazolyl, thiazolyl, $-CONR^{13}R^{14}$, alkoxy,



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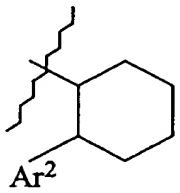

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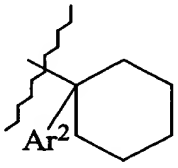

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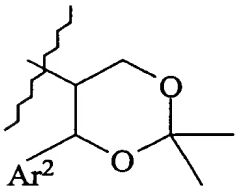

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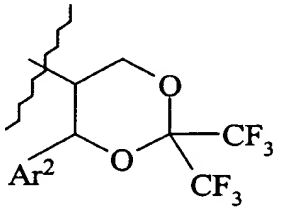


, wherein p is 0, 1 or 2 and Ar^2 is phenyl or pyridyl;

R^{10}, R^{11}, R^{12}, R^{13} and R^{14} are each independently selected from hydrogen or straight, branched, or cyclic alkyl of from 1 to 7 carbon atoms.

This invention also concerns the use of a compound of Formula I for the preparation of a medicament useful for diagnosing, preventing or treating diseases amenable to therapy by a bombesin receptor antagonist as described above.

The present invention also provides a method for treating a mammalian tumour which comprises administering to a mammal a composition comprising a tumour-inhibiting amount of a compound of Formula I, or of a conjugate of a cytotoxic agent with a compound of Formula I.

The present invention further provides a method for *in vivo* diagnostic imaging of a mammalian tissue which has cell surface bombesin receptors, which includes administering to a mammal a diagnostic imaging amount of a radiolabelled compound of the present invention, and further detecting a radiation image due to the binding of said compound to the bombesin receptors of a tissue having an abundance of cells with such receptors. In particular, this invention relates to a method for diagnosing a mammal for the presence of a mammalian

tumour which comprises administering to a mammal a diagnostic imaging amount of a compound of Formula I, and optionally detecting an image of a tissue having an abundance of cells with bombesin receptors.

A further aspect of the present invention provides a method for *in vitro* detection of a cancer cell in a mammalian tissue, which includes contacting a mammalian tissue sample with an *in vitro* diagnostic imaging amount of a compound of Formula I for a time and under conditions sufficient for binding of the compound to the cancer cell, and detecting such binding.

BRIEF DESCRIPTION OF FIGURES

Figure 1 shows the effects of Compound 1 (at 10 mg/kg, i.p. 1 h before test) on the behaviour of rats placed on the elevated plus-maze ([**A**]: % time on open arms; [**B**]: % number of open arm entries; [**C**]: Open end time; Veh.: vehicle).

Figure 2 shows the effects of Compound 1 on time spent in social interaction by rats in high light, unfamiliar conditions.

Figure 3 shows the effects of Compound 1 on the time spent by rats in the central region of the arena in the open field test (Veh.: vehicle).

Figure 4 shows the effects of Compound 1 on proceptive behaviour in female rats (Prog: progesterone).

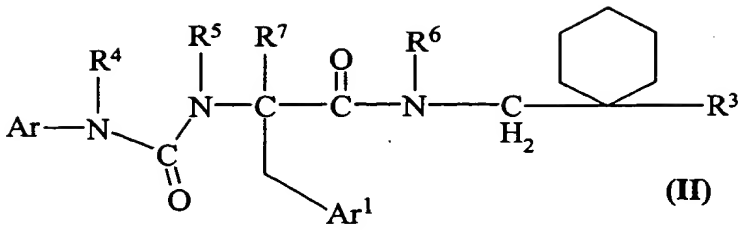
Figure 5 shows the effects of Compound 1 in the isolation-induced vocalisation model of anxiety in the guinea pig pup on proceptive behaviour in female rats.

Figure 6 shows the effect of diazepam (a) or fluoxetine (b) in the isolation-induced vocalisation model of anxiety in the guinea pig pup.

DETAILED DESCRIPTION

The compounds used in the invention are those of Formula I above.

Preferred compounds are those of Formula II



(II)

wherein

Ar is phenyl unsubstituted or substituted with 1 or 2 substituents selected from isopropyl, halo, nitro, and cyano;

R^4, R^5, and R^6 are hydrogen;

R^7 is methyl or hydrogen;

R^3 is 2-pyridyl or hydroxy; and

Ar^1 is indolyl, pyridyl, pyridyl-N-oxide, or imidazolyl.

Other preferred compounds are those of Formula I wherein

Ar is unsubstituted phenyl;

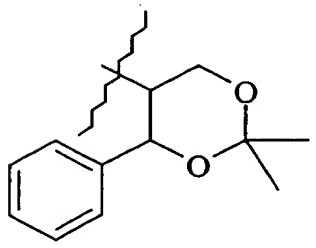
R^1 is cyclopentyl or tert-butyl;

R^4 and R^5 are hydrogen;

R^7 is methyl;

R^6 is hydrogen;

R^3 is phenyl with two isopropyl substituents, unsubstituted phenyl, or



; and

Ar^1 is indolyl.

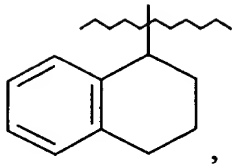
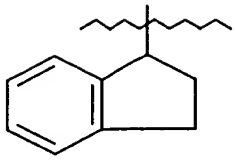
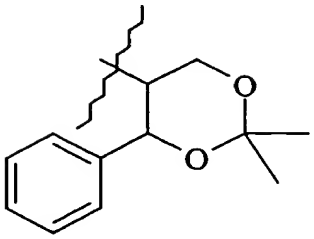
Other preferred compounds are those of Formula I wherein Ar is 2,6-diisopropyl-phenyl, 4-nitro-phenyl, and 4-cyano-phenyl;

R^4, R^5, and R^6 are hydrogen;

R^7 is methyl;

R^2 is hydrogen or cyclohexyl; and

R^3 is hydroxyl, pyridyl,

 ,  , or  .

The alkyl groups of the compounds used in the invention include straight, branched, or cyclic carbon chains of from 1 to 8 carbon atoms except where specifically stated otherwise. Representative groups are methyl, ethyl, propyl, isopropyl, n-propyl, n-butyl, iso-butyl, sec-butyl, tert-butyl, 2-methylhexyl, n-pentyl, l-methylbutyl, 2,2-dimethylbutyl, 2-methylpentyl, 2,2-dimethylpropyl, n-hexyl, and the like.

The lower alkyl groups of the compounds used in the invention comprise those having 1 to 6 carbon atoms.

The cycloalkyl groups of the compounds used in the invention comprise those having 3 to 7 carbon atoms. They may be substituted with from 1 to 3 groups selected from halogens, nitro, alkyl, and alkoxy.

The alkoxy groups of the compounds used in the invention comprise both straight and branched carbon chains of from 1 to 6 carbon atoms unless otherwise stated. Representative groups are methoxy, ethoxy, propoxy, i-propoxy, t-butoxy, and hexoxy.

The term "halogen" is intended to include fluorine, chlorine, bromine, iodine and astatine, including radionuclides thereof.

The term "Ar" is intended to include substituted or unsubstituted phenyl. The substituents include one or more substituents such as halogens, nitro, alkyl, alkoxy, and others as specified or as would occur to one skilled in the art.

The term "amine" is free amino, alkylated amines, and acylated amines.

More preferred compounds are selected from

(S) N-Cyclohexylmethyl-2-[3-(2,6-diisopropyl-phenyl)-ureido]-3-(1H-indol-3-yl)-2-methyl-propionamide;

N-Cyclohexylmethyl-2-[3-(2,6-diisopropyl-phenyl)-ureido]-3-(1H-indol-3-yl)-N-methyl-propionamide;

N-Cyclohexylmethyl-2-[3-(2,6-diisopropyl-phenyl)-1-methyl-ureido]-3-(1H-indol-3-yl)-propionamide;

2-[3-(2,6-Diisopropyl-phenyl)-ureido]-2-methyl-3-(1-oxy-pyridin-2-yl)-N-(1-pyridin-2-yl-cyclohexylmethyl)-propionamide;

2-[3-(2,6-Diisopropyl-phenyl)-ureido]-2-methyl-3-pyridin-2-yl-N-(l-pyridin-2-yl-cyclohexylmethyl)-propionamide;

2-[3-(2-tert-Butyl-phenyl)-ureido]-N-cyclohexylmethyl-3-(1H-indol-3-yl)-2-methyl-propionamide;

N-Cyclohexylmethyl-2-[3-(2,6-dichloro-phenyl)ureido]-3-(1H-indol-3-yl)-2-methyl-propionamide;

N-Cyclohexylmethyl-2-[3-(2,6-dimethoxy-phenyl)ureido]-3-(1H-indol-3-yl)-2-methyl-propionamide;

N-Cyclohexylmethyl-2-[3-(2,6-dimethylamino-phenyl)-ureido]-3-(1Hindol-3-yl)-2-methyl-propionamide;

(S) N-Cyclohexylmethyl-3-(1H-indol-3-yl)-2-methyl-2-[3-(4-nitro-phenyl)-ureido]-propionamide;

N-Cyclohexylmethyl-2-[3-(2,2-dimethyl- 1 -phenyl)propyl)-ureido]-3-(1Hindol-3-yl)-2-methyl-propionamide;

[S-(R*, R*)] 3-(1H-Indol-3-yl)-2-methyl-2-{3-[1-(4-nitro-phenyl)-ethyl]-ureido}-N-(1-pyridin-2-yl-cyclohexylmethyl)-propionamide;

N-(2,2-Dimethyl-4-phenyl-[1,3]dioxan-5-yl)-3-(1H-indol-3-yl)-2-methyl-2-[3-(1-phenyl-cyclopentylmethyl)ureido]-propionamide;

(S)-N-(2,6-Diisopropyl-phenyl)-2-[3-(2,2-dimethyl-1-phenyl-propyl)-ureido]-3-(1H-indol-3-yl)-propionamide;

(R)-N-(2,6-Diisopropyl-phenyl)-2-[3-(2,2-dimethyl-1-phenyl-propyl)-ureido]-3-(1H-indol-3-yl)-propionamide;

2-[3-(2,6-Diisopropyl-phenyl)-ureido]-N-(2,2-dimethyl-4-phenyl-[1,3]dioxan-5-yl)-3-(1H-indol-3-yl)-2-methyl-propionamide;

N-Cyclohexyl-2-[3-(2,6-diisopropyl-phenyl)-ureido]-3-(1H-indol-3-yl)-2-methyl-propionamide;

N-(2-Cyclohexyl-ethyl)-2-[3-(2,6-diisopropyl-phenyl)-ureido]-3-(1H-indol-3-yl)-2-methyl-propionamide;

2-[3-(2,6-Diisopropyl-phenyl)-ureido]-3-(1H-indol-3-yl)-2-methyl-propionamide;

2-[3-(2,6-Diisopropyl-phenyl)-ureido]-3-(1H-indol-3-yl)-2-methyl-N(3-methyl-butyl)-propionamide;

2-[3-(2,6-Diisopropyl-phenyl)-ureido]-3-(1H-indol-3-yl)-2-methyl-N(3-phenyl-propyl)-propionamide;

2-[3-(2,6-Diisopropyl-phenyl)-ureido]-3-(1H-indol-3-yl)-2-methyl-N-(1,2,3,4-tetrahydro-naphthalen-1-yl)-propionamide;

2-[3-(2,6-Diisopropyl-phenyl)-ureido]-3-(1H-indol-3-yl)-2-methyl-N-(2-phenyl-cyclohexyl)-propionamide;

2-[3-(2,6-Diisopropyl-phenyl)-ureido]-N-indan-1-yl-3-(1H-indol-3-yl)-2-methyl-propionamide;

2-[3-(2,6-Diisopropyl-phenyl)-ureido]-N-(l-hydroxy-cyclohexylmethyl)3-(1H-indol-3-yl)-2-methyl-propionamide;

2-[3-(2,6-Diisopropyl-phenyl)-ureido]-3-(1H-indol-3-yl)-2-methyl-N-(l-pyridin-2-yl-cyclohexylmethyl)-propionamide;

2-[3-(2,6-Diisopropyl-phenyl)-ureido]-3-(1H-indol-3-yl)-2-methyl-N(6,7,8,9-tetrahydro-5H-benzocyclohepten-5-yl)-propionamide;

2-[3-(2,6-diisopropyl-phenyl)-ureido]-3-(1H-indol-3-yl)-2-methyl-N-phenyl-propionamide;

N-(1-Hydroxy-cyclohexylmethyl)-3-(1H-indol-3-yl)-2-methyl-2-[3-(4-nitro-phenyl)-ureido]-propionamide;

2-[3-(4-cyano-phenyl)-ureido] 3-(1H-indol-3-yl)-2-methyl-N-(1-pyridin-2-yl-cyclohexylmethyl)-propionamide;

(S) 3-(1H-indol-3-yl)-2-methyl-2-[3-(4-nitro-phenyl)-ureido]-N-(1-pyridin-2-yl-cyclohexylmethyl)-propionamide;

(S) 3-(1H-indol-3-yl)-2-methyl- N-(1-pyridin-2-yl-cyclohexylmethyl)-2-[3-(4-trifluoromethyl-phenyl)-ureido]-propionamide;

(S) 4-(3-{2-(1H-indol-3-yl)-1-methyl-1-[(1-pyridin-2-yl-cyclohexylmethyl)-carbamoyl]-ethyl}-ureido)-benzoic acid ethyl ester;

2-[3-(2,6-Diisopropyl-phenyl)-ureido]-3-(1H-imidazol-4-yl)-N-(1-pyridin-2-yl-cyclohexylmethyl)-propionamide;

2-[3-(2,6-Diisopropyl-phenyl)-ureido]-2-methyl-N-(1-pyridin-2-yl-cyclohexylmethyl)-3-(2-trifluoromethyl-phenyl)-propionamide;

2-[3-(2,6-Diisopropyl-phenyl)-ureido]-2-methyl-3-(2-nitro-phenyl)-N-(1-pyridin-2-yl-cyclohexylmethyl)-propionamide;

(S) 3-(1H-Indol-3-yl)-N-[1-(5-methoxy-pyridin-2-yl)-cyclohexylmethyl]-2-methyl-2-[3-(4-nitro-phenyl)-ureido]-propionamide; and

N-cyclohexylmethyl-2-[3-(2,6-diisopropyl-phenyl)-ureido]-2-methyl-3-pyridin-2-yl-propionamide.

Hereinafter, (S) 3-(1H-Indol-3-yl)-N-[1-(5-methoxy-pyridin-2-yl)-cyclohexylmethyl]-2-methyl-2-[3-(4-nitro-phenyl)-ureido]-propionamide is also referred to as Compound 1.

The compounds used in the present invention can have multiple chiral centers in the above Formula I depending on their structure. In particular, the compounds used in the present invention may exist as diastereomers, mixtures of diastereomers, or as the mixed or the individual optical enantiomers. The present invention contemplates use of all such forms of the compounds.

The compounds utilized in the invention include solvates, hydrates, pharmaceutically acceptable salts, and polymorphs (different crystalline lattice descriptors) of the compounds of Formula I.

Where it is appropriate to form a salt, the pharmaceutically acceptable salts include acetate, benzenesulfonate, benzoate, bicarbonate, bitartrate, bromide, calcium acetate, camsylate, carbonate, chloride, citrate, dihydrochloride, edetate, edisylate, estolate, esylate, fumarate, gluceptate, gluconate, glutamate, glycoloylarsanilate, hexylresorcinate, hydrabamine, hydrobromide, hydrochloride, hydroxynaphthoate, iodide, isethionate, lactate, lactobionate, malate, maleate, mandelate, mesylate, methylbromide, methylnitrate, methylsulfate, mucate, napsylate, nitrate, pamoate (embonate), pantothenate, phosphate/diphosphate, polygalacturonate, salicylate, stearate, subacetate, succinate, sulfate, tannate, tartrate, theoclate, triethiodide, benzathine, chloroprocaine, choline, diethanolamine, ethylenediamine, meglumine, procaine, aluminum, calcium, lithium, magnesium, potassium, sodium, and zinc. (See also "Pharmaceutical salts" by Berge S.M. *et al.* (1997) J. Pharm. Sci. 66: 1-19, which is incorporated herein by reference.)

The terms "patient" or "subject" are intended to include a mammal, especially a human, more especially a female patient.

The methods of this invention are carried out by administering to a mammal an effective amount of a compound of Formula I, in chemotherapeutic indications a conjugate of a compound of Formula I with a cytotoxic agent, or a pharmaceutically acceptable salt thereof, to diagnose, prevent or treat any of the disorders hereinbefore described. Such effective amount will generally be from about 1 to about 300 mg per kg of subject body weight. Typical doses will be from about 1 mg to about 5 g per day for an adult of 70 kg. In addition, doses of radiolabelled compounds used depend on the specific radioactivity of the radionuclide.

In a further aspect of the present invention, there is provided a pharmaceutical composition for the treatment or prevention of the indications hereinbefore recited, said composition comprising a bombesin receptor antagonist of Formula I, together with at least one pharmaceutically acceptable carrier or excipient. For preparing pharmaceutical compositions from the compounds used in this invention, inert, pharmaceutically acceptable carriers can be either solid or liquid. Solid form preparations include powders, tablets, dispersible granules, capsules, cachets, pessaries and suppositories including vaginal suppositories. A solid carrier can be one or more substances which may also act as diluents, flavouring agents, solubilizers, lubricants, suspending agents, binders, or tablet disintegrating agents; it can also be an encapsulating material. In powders, the carrier is a finely divided solid that is in a mixture with the finely divided active component. In tablets, the active component is mixed with the carrier having the necessary binding properties in suitable proportions and compacted in the shape and size desired.

For preparing pessary or suppository preparations, a low-melting wax such as a mixture of fatty acid glycerides and cocoa butter is first melted and the active ingredient is dispersed therein by, for example, stirring. The molten homogeneous mixture is then poured into convenient sized molds and allowed to cool and solidify. Ointments are also preferred pharmaceutical compositions that can be similarly prepared from the compounds used in this invention.

The powders and tablets preferably contain 5% to about 70% of the active component. Suitable carriers are magnesium carbonate, magnesium stearate, talc, lactose, sugar, pectin, dextrin, starch, tragacanth, methyl cellulose, sodium carboxymethyl cellulose, a low-melting wax, cocoa butter, and the like.

The term "preparation" is intended to include the formulation of the active component with encapsulating material as a carrier providing a capsule in which the active component (with or without other carriers) is surrounded by a carrier which is thus in association with it. Similarly, cachets are included.

Tablets, powders, cachets, and capsules can be used as solid dosage forms suitable for oral administration.

Liquid form preparations include solutions, suspensions, and emulsions.

Sterile water or water-propylene glycol solutions of the active compounds may be mentioned as an example of liquid preparations suitable for parenteral administration. Liquid preparations can also be formulated in solution in aqueous polyethylene glycol solution.

Aqueous solutions for oral administration can be prepared by dissolving the active component in water and adding suitable colorants, flavoring agents, stabilizers, and thickening agents as desired. Aqueous suspensions for oral use can be made by dispersing the finely divided active component in water together with a viscous material such as natural synthetic gums, resins, methyl cellulose, sodium carboxymethyl cellulose, and other suspending agents known to the pharmaceutical formulation art.

Preferably the pharmaceutical preparation is in unit dosage form. In such form, the preparation is divided into unit doses containing appropriate quantities of the active component. The unit dosage form can be a packaged preparation, the package containing discrete quantities of the preparation, for example, packeted tablets, capsules, and powders in vials or ampoules. The unit dosage form can also be a capsule, cachet, or tablet itself, or it can be the appropriate number of any of these packaged forms.

As already mentioned, the compounds of Formula I can be used for the diagnosis, prevention and treatment of various disease states. Further information on such uses is provided below.

1) SEXUAL DYSFUNCTIONS

Sexual dysfunctions include disturbances in the sexual response cycle and pain associated with sexual arousal or intercourse. Symptoms include reduced libido, anorgasmy, vaginismus, dyspareunia and impotence.

Physical causes of sexual dysfunctions include increasingly frequent disorders such as obesity, depression, cardiovascular disorders, diabetes mellitus, endocrine disorders and neurologic disorders (e.g. multiple sclerosis, neuropathies). In addition several widely used drugs are

known to cause sexual dysfunction, including antihypertensives, histamine H_2 receptor antagonists, sedatives and anxiolytics, selective serotonin reuptake inhibitors and tricyclic antidepressants.

Hypoactive sexual desire disorder is the most common type of sexual dysfunction in women, occurring in 20 % of women and in 10 % of men (The Merck Manual, 17th edition, 1999), causing marked distress and interpersonal difficulties.

Treatment of sexual dysfunctions is directed at removing or alleviating the underlying cause, but the aetiology of sexual dysfunctions is complex and varies greatly: in addition to the physical causes mentioned, psychological and situational factors e.g. stressful life situations are often involved. Therefore there was no standard treatment available so far.

In addition, sexual dysfunctions can induce anxiety, fear or guilt, frustration or resentment, and conflict in a relationship, which in turn may reinforce the symptoms.

It is known that a decrease in serotonin (5HT) activity can result in increases in sexual activity. Bombesins are present in the urinogenital tract, and in areas of the hypothalamus known to change sexual function, where bombesin receptors are closely associated with 5HT-neurons which they activate (Battey J. *et al* (1991), Trends Neurosci. **14**: 524; Woodruff G.N. *et al* (1996) *in*: Neuropeptides: Basic and Clinical Advances, eds J.N. Crawley and S. McLean (The New York Academy of Sciences, New York) p. 223).

BB_1 and BB_2 receptors are present in the medial pre-optic nucleus (Ladenheim *et al.* (1992) Br. Res. **593**: 168-178). This area is one of the most important regions of the brain controlling sexual function in male rats. Additionally bombesin-like peptides have been detected in the seminal fluid. Thus in both brain centres and the genital system may bombesin receptor antagonists combat disturbances of the sexual physiology.

NMB receptors are also found in the ventromedial hypothalamus (VMH) (Ladenheim *et al.*, 1992, Br. Res. **593**: 168-178). This area is one of the most important regions of the brain controlling sexual function in female rats. Alpha-melanocyte stimulating hormone (α-MSH) secretion in areas of the CNS such as the VMH increases sexual receptivity. It has been shown that bombesin is able to reduce α-MSH secretion (Toney *et al.*, Br. Res. 1992, **598**: 279-285). This antagonism of bombesin-induced inhibition of α-MSH would be expected to increase sexual receptivity.

The compounds of the instant invention are useful in the treatment of male and especially female sexual dysfunctions, as demonstrated by means of the following pharmacological procedures.

a) Sexual behavior of male rats:

Methods: A group of male Wistar rats were tested 3 times for the occurrence of mounting behavior and were accordingly allocated to vigorous, intermediate, and sluggish groups for testing. Each rat was observed on 6 separate occasions (3 times following vehicle injections and 3 times following administration of 10 mg/kg i.p. Compound 1) for a maximum of 30 min. Parameters measured were: number of mounts, mount latency, number of intromissions and ejaculatory latency.

Results: (Table 1) There was no effect of drug treatment on the behavior of intermediate and sluggish rats (data not shown). However there was an increase in ejaculatory latency ($p < 0.05$) and mount latency (non-significant trend) of the vigorous rats.

Table 1: Behavioral measures* in vigorous male rats

	Number of mounts	Mount latency (s)	Number of intromissions	Ejaculatory latency (s)
Vehicle	12.2 ± 1.3	64.0 ± 25.5	12.4 ± 0.9	454.1 ± 51.9
Compound 1 10 mg/kg i.p.	13.8 ± 2.2	122.9 ± 33.8 (NS $p = 0.16$)	12.5 ± 1.1	807.3 ± 94.4 ($p < 0.05$)

*Overall means of 3 separate tests performed over 3 weeks.

Compound 1 (10 mg/kg i.p.) transiently reduced male sexual function. This effect was only transient however, as the effect was due to a large reduction in function on first testing with subsequent injections having little effect on parameters of sexual behaviour.

b) Sexual receptivity in female rats:

Methods: 20 female Wistar rats were ovariectomised and allowed to recover for 2 weeks; 48 h before each test the rats were injected with oestradiol benzoate (10 µg/rat). Testing was done every second week and the lordotic quotient (LQ: % lordotic response to 10 mounts from a vigorous male) calculated. Females were divided into receptive (those having LQ>50%) and non-receptive (those having LQ<50%) groups. Over a period of 4 weeks all rats received both vehicle and Compound 1 (10mg/kg i.p.).

Results: (Table 2) Receptive female rats were not affected by either vehicle or Compound 1 treatment, indicating that Compound 1 has no inhibitory effect on female sexual behavior. In non-receptive rats, Compound 1 significantly increased receptivity (both relative to vehicle and to pre-treatment scores).

Table 2: Lordotic quotient in female rats (%)

	Receptive females			Non-receptive females		
	n	**Pre-treatment**	**Post-treatment**	**n**	**Pre-treatment**	**Post-treatment**
Vehicle	8	74.4 ± 4.6	82.5 ± 4.1	12	2.5 ± 1.3	11.7 ± 7.3
Compound 1	5	73.0 ± 9.5	88.0 ± 8.0	10	16.5 ± 6.1	59.5 ± 10.1 * +

* $p < 0.0$ 1 compared to pre-treatment + $p < 0.01$ compared to vehicle

Compound 1 (10 mg/kg i.p. 60 min) stimulates the sexual activity of non-receptive female rats.

c) Sexual proceptivity in female rats

Methods: Ovariectomised female Sprague-Dawley rats (180-200g, from Charles River) were housed in groups of 6 in a reversed lighting system of 12 h light:dark (lights off 5.00-17.00h). Two weeks after ovariectomy they were used for tests. The experiments started at least 5 h into the dark period. The test was carried out in a circular arena of 90-cm diameter, surrounded by a 30-cm high wall. Two small cages with wire-mesh front (15x15 cm) are fixed into the wall such that the front of the cage is flush with the wall and the 2 cages are opposite each other. They contain two stimuli animals, an intact sexually experienced male and a receptive female (ovariectomised, primed with 5 μg oestradiol 48 h before the test and 0.5 mg progesterone 4 h before the test). Animals were adapted to the apparatus (in the absence of stimuli animals) for 10 min on 2 consecutive days prior testing. During the 10-min test, time spent investigating each stimulus animal is taken. The difference in the percentage of time spent investigating male minus female was calculated, out of the total time spent investigating stimuli animals. The arena was thoroughly cleaned between animals. The position of the male/female stimuli boxes was randomised between animals, in order to avoid place preference. Sexually naïve animals were used in this test. Forty eight hours before tests, the animals were primed with 5

μg oestradiol benzoate dissolved in corn oil and injected subcutaneously (s.c.). Progesterone (0.5 mg/0.1 ml) was dissolved in corn oil and administered s.c. 4 h before test, as a positive control. Compound 1 dissolved in 100% β-cyclodextrin and then diluted with saline to a final solution of 50% 2-hydroxypropyl-β-cyclodextrin was administered intraperitoneally (i.p.) at a dose of 10 mg/kg, in a dosing volume of 1 ml/kg, 1 h before tests. Progesterone (0.5 mg/0.1 ml) was dissolved in corn oil and administered s.c. 4 h before test, as a positive control.

Results: Compound 1 dose-dependently (3 - 10 mg/kg) increased the percentage of time spent investigating the male stimulus, with an MED of 100 mg/kg (see Figure 4). The effect of this dose was similar to the effect of progesterone. (*P<0.05, **P<0.01 Kruskall-Wallis followed by Mann-Whitney test, *vs* vehicle).

2) ANXIETY, PANIC ATTACKS AND SOCIAL PHOBIA

Anxiety is a very commonly observed symptom, for which benzodiazepines are the primary treatment agents. Chlordiazepoxide, diazepam, oxazepam, lorazepam, prazepam and alprazolam are most commonly used for this purpose in the United States. However anxiolytic benzodiazepines may also cause sedation, they have muscle-relaxant, sedative-hypnotic, and amnestic side effects; they also tend to potentiate the effects of alcohol. Some tolerance to their effects may develop, withdrawal after chronic use frequently induces rebound anxiety, and long-term use of benzodiazepines, particularly with escalating doses, can lead to dependence. Therefore there is a need for anxiolytic treatments with a reduced dependence liability.

Recent findings may suggest a role of bombesin-like peptides in stress and anxiety (Plamondon H. *et al.* (1996) Soc. Neurosci. **22**: Abstract 181.13): antisense oligonucleotides to mRNA for GRP receptors and NMB receptors were infused i.c.v. in rats over 2 days, resulting in a reduction of bombesin binding site density in the brain, as measured by receptor autoradiography. Rats treated with the antisense oligonucleotides spent significantly more time on the anxiogenic fields of an elevated plus maze, or of a trough-tunnel oval maze, reflecting an anxiolytic effect of treatment, as compared to control animals.

The compounds of the instant invention are useful in the treatment of anxiety and of panic as demonstrated by means of pharmacological procedures, some of which are exemplified below.

Animals and Drug administration

- Male Hooded Lister rats (200-250 g) or male TO mice (20-25 g) are housed in groups of six. Common marmosets (*Callithrix jacchus*) weighing 280-380 g are housed in pairs. All animals are housed under a 12-hour light/dark cycle, with food and water *ad libitum*.

- Unless otherwise specified, drugs are administered intraperitoneally (i.p.) or subcutaneously (s.c.) 40 min before the test in a volume of 1 ml/kg for rats and marmosets and 10 ml/kg for mice.

a) Mouse light/dark box test

The apparatus is an open-topped box, 45 cm long, 27 cm wide, and 27 cm high, divided into a small (2/5) and a large (3/5) area by a partition that extends 20 cm above the walls (Costall B. *et al.* (1989) Pharmacol. Biochem. Behav. **32**: 777-785).

There is a 7.5 x 7.5 cm opening in the center of the partition at floor level. The small compartment is painted black and the large compartment white. The white compartment is illuminated by a 60-W tungsten bulb. The laboratory is illuminated by red light. Each mouse is tested by placing it in the center of the white area and allowing it to explore the novel environment for 5 min; the time spent in the illuminated site is measured (Kilfoil T. *et al.* (1989) Neuropharmacol. **28**: 901-905).

b) Rat elevated X-maze test

Methods: A standard elevated X-maze (Handley S.L. *et al.*(1984) Naunyn-Schiedeberg's Arch. Pharmacol. **327**: 1-5) was automated as described by Field *et al.* (1991) Br. J. Pharmacol. **102** Suppl.: 304P. The animals are placed on the center of the X-maze facing one of the open arms. For determining anxiolytic effects, the entries and time spent on the end half sections of the open arms is measured during the 5-minute test period (Costall *et al.* (1989) Br. J. Pharmacol. **96** Suppl.: 312P).

Results: (Figure 1) In rats given Compound 1 at the dose of 10 mg/kg i.p. 60 min before the test, there was a significant increase in % time spent on the open arms of the maze, and also in the amount of time spent exploring the ends of the open arms, as compared with rats receiving only the vehicle.

c) Marmoset human threat test

The total number of body postures exhibited by the animals towards the threat stimulus (a human standing approximately 0.5 m away from the marmoset cage and staring into the eyes of the marmoset) is recorded during the 2-minute test period. The body postures scored are slit stares, tail postures, scent marking of the cage/perches, piloerection, retreats, and arching of the back. Each animal is exposed to the threat stimulus twice on the test day before and after drug treatment. Drug treatment is carried out s.c. at least 2 h after the first (control) threat; the pretreatment time for each compound is 40 min. The difference between the two scores is analyzed using one-way analysis of variance followed by Dunnett's t-test.

d) Rat conflict test

Rats are trained to press levers for food reward in operant chambers. The schedule consists of alternations of four 4-minute unpunished periods on variable interval of 30 s signalled by chamber lights on, and three 3-minute punished periods on fixed ratio 5 (by footshock concomitant to food delivery) signalled by chamber lights off. The degree of footshock is adjusted for each rat to obtain approximately 80 % to 90 % suppression of responding in comparison with unpunished responding. Rats receive saline vehicle on training days.

f) Rat social interaction test

Methods: The social interaction arena is circular (diameter 70 cm) and made of white perspex with walls 30 cm high. The arena is lit by a bright light source (350 lux) located directly above the arena. A camera, linked to a video recorder in an adjacent room, is also located directly above the arena to allow the test sessions to be recorded for later analysis.

Rats are allocated to a partner on the basis of body weight, so that members of a pair do not differ by more than 10 g. Both rats in each pair are injected *i.p.* with either vehicle (n = 10) or various doses of the test compound (n = 10/group) 60 min prior to being placed into the arena for a period of 5 min. Time spent in active social investigation (sniffing, following and grooming the partner) is recorded by an observer blind to drug treatment. Testing is performed between 10:00 and 14:00 h in an order randomized for drug treatment, and the arena thoroughly wiped after each trial. Data are analyzed by one-way ANOVA, followed by post-hoc Duncan's tests for individual differences.

Results: (Figure 2) Compound 1 significantly increased levels of social interaction of the rats at both 3.75 and 7.5 mg/kg [$F(2,27) = 7.2$, $p<0.01$]. In terms of percent increase, the two doses were similar. There was no effect on locomotor activity, thereby indicating the effect to be selective to levels of social interaction.

g) Rat open field test

Methods: The open field arena is circular (diameter 70 cm) and made of white perspex with walls 30 cm high. The arena is lit by a bright light source (350 lux) located directly above the arena. A camera, linked to a video recorder in an adjacent room, is also located directly above the arena to allow the test sessions to be recorded for later analysis. The floor is divided into an inner and outer circle by a line. Sixty minutes after dosing, each rat is placed in the center of the arena for 5 min and left to explore. An observer blind to drug treatment scores the time spent near the perimeter of the arena, and the time spent in the inner area of the arena. The animal is considered to be in the inner arena when all four paws are in the area, and likewise, in the perimeter, when all four paws are in the outer circle. Time spent in the inner area is expressed as a percent of total time in the inner and outer areas, and analyzed by one-way ANOVA followed by post-hoc Duncan's tests for individual differences.

Results: (Figure 3) Compound 1 at 3.75 mg/kg *i.p.* significantly increased the percent of time spent in the inner area of the arena as compared to control animals, reflecting an anxiolytic effect of treatment.

h) Isolation-induced vocalisation model of anxiety in the guinea pig pup

Animal models of psychiatric diseases commonly use rats or mice as experimental animals. However, guinea pigs here are more relevant experimental animals, since they possess central $5HT_{1D}$ receptors, similar to humans. Guinea pig vocalisations evoked by transient maternal separation is a test model for affective behaviour. Also, it is known that compounds capable of inhibiting isolation calls in this guinea pig model of anxiety are predictive of clinical effect.

Methods: Distress vocalisations of guinea-pig pups (2-14 days old) were quantified in a 5-min isolation period, after which they were reunited with their mothers and littermates. The test cage consisted of a sound-attenuating box with a white interior and white illumination. The vocalisations were recorded on DAT-tape by means of a microphone and a DAT recorder. Pups were first selected using the criterion of emitting a minimum of 500 vocalisations after

three pre-tests on three consecutive days. Diazepam and the serotonin uptake inhibitor fluoxetine were included as positive controls. Each pup received the test compound (Compound 1, 1-30 mg/kg, i.p. in cyclodextrine 50%, diazepam 0.1-1 mg/kg, s.c. in CMC, or fluoxetine 1-10 mg/kg, s.c. in water vehicle), and was returned to the home cage for 30 min before maternal separation. Diazepam (1 mg/kg) was tested in parallel every day as a positive control. The difference in the number of calls emitted before and after treatment was counted and analysed by means of a paired *t* test for each group. Percentage of reduction in the number of calls was analysed using a Kruskall-Wallis test followed by a Mann-Whitney test between vehicle and different doses.

Results: Compound 1 significantly reduced the isolation-induced vocalisations, at all the doses studied. A single administration of Compound 1, 30 min before the test, dose-dependently (1-30 mg/kg, i.p.) increased the percentage of reduction in the number of calls, with an MED of 10 mg/kg. (Figure 5: Results are expressed as mean percentage of reduction ± SEM. *P<0.05 vs vehicle group, Kruskall-Wallis test followed by Mann-Whitney test).

This effect was superior to the one observed for diazepam (MED = 1 mg/kg; Figure 6). Higher doses of this compound were not included in the study because of sedation. Fluoxetine also reduced significantly the number of calls, with an MED of 10 mg/kg (Figure 6).

It appears that both anxiolytic and antidepressant drugs reduce isolation calls emitted by guinea pigs. The bombesin antagonist Compound 1 was as effective as these compounds, indicating a potential for this class of compounds as novel anxiolytic/antidepressant agents.

3) PULMONARY HYPERTENSION

Hurel S.J. *et al.* (Lancet (1996) **348**: 1243) have shown that infusion of a GRP receptor antagonist to a patient suffering from pulmonary hypertension was followed by a decrease in the pulmonary systolic pressure.

The compounds of the instant invention are useful in the treatment of pulmonary hypertension as demonstrated by means of standard pharmacological procedures.

4) LUNG REPAIR AND LUNG DEVELOPMENT DISORDERS

Several studies have emphasized the role of GRP and the GRP receptor in lung repair after injury and in lung development (Spurzem J.R. *et al.* (1997) Am. J. Respir. Cell. Mol. Biol . **16**: 209-211; Wang D. . *et al.* (1996) Am. J. Respir. Cell. Mol. Biol. 14: 409-416; Spindel E.R.,

Ibidem 14: 407-408). Also, lung injury, including that induced by smoking, leads to increased levels of pulmonary bombesin-like peptides.

Findings by Cutz E. et al. (Pediatrics (1996) **98**: 668-72) may suggest that maternal smoking potentiates hyperplasia of the pulmonary neuroendocrine cells (as measured by the percentage of airway epithelium immunoreactive for bombesin) in the lungs of infants who die of sudden infant death syndrome (SIDS) and that a dysfunction of these cells may contribute to the pathophysiology of SIDS.

The compounds of the instant invention are useful in the treatment of lung repair and lung development disorders as demonstrated by means of standard pharmacological procedures.

5) CANCER TREATMENT

The invention also relates to a method for treating cancer which comprises administering to a patient or a subject, particularly a mammal, more particularly a human, an effective amount of a compound of Formula I, optionally conjugated with a cytotoxic agent. The method is particularly useful in cancers where tumour cells have a cell surface bombesin receptor, including certain prostate or pancreatic cancers.

When a directly labelled compound of Formula I is used for therapeutic purposes, preferably a halogen substituent of Ar as a radionuclide is used. Preferably halogen radionuclides employed for therapy are β-emitting or α-emitting radionuclides. The preferred halogen substituents of Ar for treating cancers include ^{131}I, ^{211}At, ^{76}Br and ^{77}Br, ^{131}I being particularly preferred.

Compounds of Formula I where Ar is substituted by a radionuclide halogen can easily be prepared via electrophilic aromatic substitution of a corresponding non-radioactive compound wherein Ar is substituted by a halide or an activating group. Such a halide is preferably Br or I. Preferred activating groups include tributyl-tin, trimethylsilyl, t-butyldimethylsilyl, and the like.

Conjugation of a compound of Formula I with a cytotoxic agent is especially preferred when, in the compound of Formula I, R^3 is hydroxy or amino. In such a case, the compounds of the invention may conveniently be linked to a cytotoxic agent, using a bifunctional moiety like glutaric acid or the like to form a conjugate. Suitable cytotoxic agents include compounds such as doxorubicin, anticancer chemotherapy compounds such as those described in The Merck Index, 12th edition, 1996, p. MISC-10.

The use of a conjugate of a compound of Formula I with a radionuclide is also provided by the instant invention; preferred radionuclides used for radiotherapy emit an α or β particle; they include ^{188}Re, ^{131}I, ^{211}At, ^{212}Pb, ^{212}Bi, ^{76}Br, ^{77}Br, and the like (for examples, The Merck Index, 12th edition, 1996, page MISC-93). Said conjugates may be prepared using conventional methods. For example, radionuclides such as ^{188}Re can be linked to a compound of Formula I using a bifunctional chelating agent such as trisuccin (Safavy A. *et al.* (1993) Bioconj. Chem. **4**: 194-8) according to a process adapted from Safavy A. *et al.* in Cancer (1997) **80** (Suppl): 2354-9. The conjugate may take the form of a compound which is cleaved to release the cytotoxic agent on entry into the tumour cells. Compounds that are rapidly transformed *in vivo* to yield the parent compound of the above formulae, e.g. by hydrolysis upon entry into a target cell, are preferred.

A method of the present invention for treating a mammalian tumour includes administering to a mammal a composition including a tumour-inhibiting amount of at least one compound of the present invention. Such a tumour-inhibiting amount is an amount of at least one of the subject compounds which permits sufficient tumour localization of the compound to diminish tumour growth or size. This dosage can range from about 0.1 mmol/kg body weight to about 500 mmol/kg body weight. A preferred dosage is about 5 to about 50 mmol/kg body weight.

The amount of radioactivity administered can vary depending on the type of radionuclide. However, with this in mind the amount of radioactivity which is administered can vary from about 1 millicurie (mCi) to about 800 mCi. Preferably, about 10 mCi to about 600 mCi is administered. Moreover when considering the dosage, the specific activity of the radioactive compound should be taken into consideration. Such a specific activity is preferably very high, e.g. for ^{123}I-labeled compounds the specific activity should be at least about 1,000 Ci/mM to about 50,000 Ci/mM. More preferably the specific activity for ^{123}I-labeled compounds is, e.g., about 10,000 Ci/mM to about 22,000 Ci/mM.

a) Prostate cancer

Bombesin specifically induces intracellular calcium mobilization via GRP receptors in human prostate cancer cells (Aprikian A.G. *et al.*(1996) J. Mol. Endocrinol **16**: 297-306). This may suggests that the bombesin family of neuropeptides could play a regulatory role in the biology

of prostate cancer. The use of antibodies raised against bombesin inhibited the growth of a prostatic carcinoma cell line (Hoosein N.M., (1993) Cancer Bull. **45**:436-441).

The compounds of the instant invention are useful in the diagnosis and treatment of prostate cancer as demonstrated by means of standard pharmacological procedures.

b) Pancreatic cancer

Normal and tumour pancreatic cells contain a specific GRP receptor that is expressed more on malignant pancreatic tissues (Hajri A. *et al.*(1996) Pancreas **12**: 25-35). Bombesin-like peptides may stimulate proliferation of human pancreatic cancer cells (Wang Q.J. *et al.* Int. J. Cancer (1996) **68**: 528-34). As a consequence a bombesin receptor antagonist may be used to treat pancreatic cancers. Furthermore, a radiolabelled bombesin receptor antagonist may be used to treat pancreatic cancers.

The compounds of the instant invention are useful in the treatment of pancreatic cancer as demonstrated by means of standard pharmacological procedures.

6) HEPATIC PORPHYRIA

The major clinical manifestation of hepatic porphyrias are neurologic symptoms, including abdominal pain, neuropathy, and mental disturbances. It is believed that the neurologic symptoms are caused by an increase of a few gastrointestinal and neurotransmitter polypeptides, including GRP, in the systemic circulation during the acute phase of the disease (Medenica R. *et al.* (1997) Cell mol. Biol. **43**: 9-27). Treatment with bombesin receptor antagonists may thus reduce the effects of those polypeptides that bind to bombesin receptors, and alleviate the symptomatology of acute porphyria.

The compounds of the instant invention are useful in the treatment of hepatic porphyria as demonstrated by means of standard pharmacological procedures.

7) VISCERAL PAIN

The compounds used in the present invention are useful in visceral pain, as demonstrated by the following pharmacological procedure:

Irritated colon in rats

Under anesthesia, rats are tracheotomized and a carotid artery is cannulated for monitoring of blood pressure. Forty-five minutes after intracolonic injection of 0.6 % acetic acid, 3 colonic

distensions (75 mmHg; 30 s) are performed. The colonic pain is evaluated by measurement of the fall of blood pressure after these colonic distensions. Then the test compounds are administered subcutaneously, and 30 min later, 6 colonic distensions are performed again. The percentage of antinociception is computed by comparison of the blood pressure decrease in the pretreatment period with that of the post-treatment period.

Using this procedure, Compound 1 at 10 mg/kg produced an antinociception of 57 %.

8) GASTROINTESTINAL SECRETORY DISTURBANCES

GRP has proved to be a particularly valuable tool in detecting disturbances of gastric secretory function, including those associated with duodenal ulcer disease and *Helicobacter pylori* infection (McColl K.E. *et al.* (1995) Aliment. Pharmacol. Ther. **9**: 341-7). As a consequence, a radiolabelled bombesin receptor antagonist may be useful to diagnose these conditions. Other gastrointestinal functions such as gallbladder contraction, pancreatic secretion and gastro-esophageal motility are subject to regulatory controls by GRP, and a radiolabelled bombesin receptor antagonist may be useful to diagnose these conditions.

The compounds of the instant invention are useful in the treatment of gastrointestinal secretory disturbances as demonstrated by means of standard pharmacological procedures.

9) EMESIS

Bombesin is present in high concentrations in the skin of frogs. As part of a defense reaction, Amphibia secrete emetic substances when swallowed by a predator.

In mammals, bombesin is widely distributed in the GI tract where it causes changes in gastric motility and secretion.

Bombesin receptor antagonists of the invention may decrease retching and vomiting and thus be effective in the treatment of emesis, in particular in patients receiving anticancer agents.

The following pharmacological procedure may be used to demonstrate the efficacy of the compounds of the invention in the treatment of emesis.

Antagonism of cisplatin-induced emesis in ferrets

The antiemetic properties of the compounds used in the present invention can be evidenced *in vivo* on cisplatin-induced emesis in ferrets, as compared to control animals, using the following procedure. Cisplatin (30 mg/kg) is injected by the i.v. route. Investigational compounds generally are injected by a parenteral route 30 min before administration of cisplatin,

and again 45 min after the injection of the emetogen. Following administration of the emetic substance, ferrets are observed continuously in individual cages during a 5-hour period. In each animal, the number of retching and vomiting episodes is counted for the duration of the observation. Results represent the mean ± S.E.M. for each treatment group. The significance of difference between treatment with an invention compound and with the vehicle (PEG 200; 0.5 ml/kg) is assessed using a one-way analysis of variance (ANOVA) followed by a Student's t-test.

10) ANOREXIA

Bombesin causes a decrease of glucose intake in mice. In mice lacking the GRP receptor, bombesin no longer showed this effect (Hampton L. *et al*, Proc. Natl.Acad. Sci. USA, **95**: 3188-92, 1998). Bombesin receptor antagonists used in the present invention may increase feeding behavior, and thus be effective in the treatment of anorexia, such as the anorexia of cancer patients.

Orexigenic effects of the compounds used in the instant invention can be evidenced *in vivo* by measuring the food intake and the bodyweight gain of mice, as compared to control animals.

11) PAIN

The compounds of the invention are useful in the treatment of pain as demonstrated by means of the following pharmacological models:

a) Carrageenan-induced hyperalgesia and allodynia in the rat

Carrageenan (100 µl of 20 mg/ml) is administered into plantar surface of a hind paw. Hyperalgesia and allodynia to peripheral thermal and mechanical stimulation is measured.

b) Ovario-hysterectomy model of surgical pain

Female rats are anesthetized with isoflurane (5% for induction, 2% for maintenance of anesthesia) and 1:4 O_2/NO_2 mixture, which is maintained during surgery via a nose cone. Animals are then placed on a homeothermic blanket for the duration of the procedure. Ovario-hysterectomy is performed via a midline abdominal incision (2 cm in length) in the *finea alba*. The ovarian ligaments and the cervix are ligated with 5.0 silk, using single clamp technique.

Four simple interrupted sutures (5.0 silk) are placed in the abdominal wall. The skin is closed with four wound clips and the wound is treated with topical antibiotics.

The animals are tested post-operatively for signs of visceral nociception. Immediately after surgery, animals are placed in an individual plexiglass cage and abdominal postures are recorded in 30 min batches. Postures scored are humpbacked position, contraction of the muscles of the abdomen associated with inward movements of the hind limb, stretching of the body, and squashing of the lower abdomen against the floor. Animals are also tested for signs of referred hyperalgesia and allodynia in the hind paws.

c) Diabetes-induced model of neuropathic pain

Diabetes is induced in rats (250 - 300 g) by a single i.p. injection of streptozocin (50 mg/kg) as described previously (Courteix *et al.*, 1993, Pain **53**: 81-88). Control animals receive a similar administration of isotonic saline.

d) Chronic constriction injury model of neuropathic pain

The chronic constriction injury (CCI) is induced as described previously by Bennett and Xie, in Pain **33**: 87-107 (1988). Briefly, rats (175-200g) are anesthetized with sodium pentobarbital 60 mg/kg i.p. The common left sciatic nerve is exposed at the level of the middle of the thigh by blunt dissection through *biceps femoris*, and proximal to the sciatic trifurcation, 4 ligatures (4.0 braided silk) are tied loosely around it with about 1 mm spacing. The muscle is then closed in layers.

Measurement of pain thresholds may be carried out according to several procedures, including:

1) Thermal hyperalgesia

Thermal hyperalgesia is assessed, before and at various time points after drug administration, using the Ugo Basile Plantar test in rats, following a modified method of Hargreaves *et al.* (1988) Pain **32**: 77-88. Animals are habituated to the apparatus consisting of three individual perspex boxes on an elevated glass table. A mobile radiant heat source is located under the table and focused onto the desired paw. Paw withdrawal latencies are recorded. There is an automatic cut off point of 22.5 s to prevent tissue damage.

2) Static allodynia

Mechanical hypersensitivity can be measured using Semmes-Weinstein von Frey hairs. Animals are placed into wire mesh bottom cages allowing access to the underside of their paws; they are habituated to this environment prior to the start of the experiment. Mechanical hypersensitivity is tested by touching the plantar surface of the animals' right hind paw with von Frey hairs in ascending order of force (0.7, 1.2, 1.5, 2, 3.6, 5.5, 8.5, 11.8, 15.1 and 29 g) for up to 6 s. Once a withdrawal response is established, the paw is re-tested, starting with the next descending von Frey hair, until no response occurs. The highest force of 29 g lifts the paw as well as eliciting a response, thus represents the cut off point. The lowest amount of force (in g) required to elicit a response is recorded as the paw withdrawal threshold (PWT).

11) DIAGNOSTIC

The present invention provides a method for *in vivo* diagnostic imaging of a mammalian tissue which has cell surface bombesin receptors which includes administering to a mammal a diagnostic imaging amount of a compound of the present invention and detecting an image of a tissue having an abundance of cells with bombesin receptors.

In particular, the present invention provides methods for detecting certain types of cancer, e.g. prostate and pancreatic cancers. The compounds used in the present invention bind to a cell surface bombesin receptor and exhibit intense cell specificity and affinity for the above cancerous cells and for cells having bombesin receptors.

In one embodiment, the present invention is directed to a method for detecting a mammalian tumour which includes administering to a mammal a diagnostic imaging amount of a compound of Formula I above, and observing retention of the compound in a tissue of the mammal.

The present invention further provides a method for diagnosing cancers using a labelled, preferably a bombesin receptor antagonist of Formula I labelled with an appropriate radionuclide suitable for imaging. Examples of radionuclides suitable for imaging are described by Michelot J.M. *et al.* (1991) in J. Nucl. Med. **32**: 1573-80. Preferred radionuclides for diagnostic imaging do not emit a particle, e.g. an α or β particle.

When used for diagnostic imaging a halogen substituent of Ar as a radionuclide may be used. Moreover said halogen radionuclide groups which are preferably used for diagnostic imaging are mainly γ-emitting radionuclides, which can be detected by radioimaging procedures, e.g.

by scintigraphic imaging. Such γ-emitting radionuclides emit radiation which is sufficently penetrating to be detected through tissues. Preferred halogen substituents of Ar groups for diagnostic imaging include ^{123}I, ^{124}I, ^{125}I, ^{131}I, ^{18}F, ^{76}Br and ^{77}Br. More preferred groups for diagnostic imaging include ^{123}I, ^{125}I and ^{18}F. ^{123}I is especially preferred for diagnostic imaging.

The present compounds can bind to a specific cell receptor prevalent on certain types of cancer cells. Such cancer cells include pancreatic carcinoma, prostate adenoma and related cells. An example of the cell receptor to which the present compounds bind is a cell surface bombesin receptor.

The binding characteristics of the compounds used in the present invention have been evaluated in receptor binding assays, as described in WO 98/07718.

According to the present invention, a method for *in vivo* detecting a mammalian tumour or a tissue containing cell surface bombesin receptor includes administering to a mammal a composition including a diagnostic imaging amount of at least one of the present compounds. Such a diagnostic imaging amount is a dosage of at least one of the subject compounds which permits sufficient tumour or tissue localisation of the compound to allow detection of the tumour or tissue. This dosage can range from about 1 μg to about 1 g of the compound per litre which can be administered in doses of about 1 ng/kg body weight to about 10 μg/kg body weight. Preferred dosages of the present compounds are in the range of about 10 ng to about 2 μg/kg for diagnostic imaging. Moreover, for diagnostic imaging the amount of radioactivity administered should be considered. Preferably about 0.1 mCi to about 20 mCi of radioactive compound is administered.

As described herein a tumour or tissue labelled with one or more of the present compounds can be detected using a radiation detector, e.g. a γ-radiation detector. One such procedure utilises scintigraphy. Tomographic imaging procedures such as single photon emission computed tomography (SPECT) or positron emission tomography (PET) can also be used to improve visualisation.

In another embodiment, the present invention provides a method for *in vitro* detection of a cancer cell in a mammalian tissue sample, which includes contacting a mammalian tissue sample with an *in vitro* diagnostic imaging amount of a compound of any one of Formulae I or II for a time and under conditions sufficient for binding of the compound to a cell surface bombesin receptor on the cancer cell, and detecting such binding. Samples can be collected by

procedures known to the skilled artisan, e.g. by collecting a tissue biopsy or a body fluid. Samples can be sectioned, e.g. with a microtome, to facilitate microscopic examination and observation of bound compound. Samples can also be fixed with an appropriate fixative either before or after incubation with one of the present compounds to improve the histological quality of sample tissues. Conditions sufficient for binding of the compound to a cell surface bombesin receptor on the cancer cell include standard tissue culture conditions, i.e. samples can be cultured *in vitro* and incubated with one of the present compounds in physiological media. Such conditions are well known to the skilled artisan. Alternatively, samples can be fixed and then incubated with a compound of the present invention in an isotonic or physiological buffer. An amount of at least one of the present compounds for *in vitro* detection of a cancer cell can range from about 1 ng/l to about 1000 µg/l. A preferred amount is about 1 µg/l to about 100 µg/l.

When the present compounds are used for *in vitro* diagnosis of cancer, a substituent of Ar as a radionuclide may be used. Preferable substituent radionuclides for *in vitro* diagnosis of cancer include ^{125}I, ^{18}F, $-^{14}COOH$, $-^{14}CH^3$, ^3H and the like. For detection of cellular binding of one of the present compounds, samples can be incubated in the presence of a selected compound, then washed and counted in a standard scintillation counter. Alternatively samples can be dipped in photographic emulsion and the signal detected under light microscopy after several days, as exposed silver grains.